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New records of bryosymbiotic pyronemataceous fungi (Ascomycota) from Mexico

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Abstract. *Neottiella albocincta* (Berk. & M.A. Curtis) Sacc., *N. rutilans* (Fr.) Dennis, *Octospora maireana* (Seaver) Yei Z. Wang, and *O. texensis* Benkert are reported for the first time from Mexico. These species, all belonging to the family Pyronemataceae, have close relationships with several species of mosses such as those in the genera *Archidium* Brid., *Atrichum* P. Beauv., and *Polytrichum* Hedw. The specimens were collected in montane cloud forest, riparian and *Quercus* forest in the states of Puebla and Tamaulipas. With the addition of these species, the number of bryosymbiotic fungi in Mexico increases to five.

Key words. *Neottiella*, *Octospora*, Pezizales, Pyronemataceae, Puebla, Tamaulipas

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INTRODUCTION

Bryosymbiotic fungi are organisms directly associated with gametophytes and sporophytes of several species of bryophyte plants (Quintero et al. 2020). About 350 mushroom species with around 90 genera are known, mostly ascomycetes, many of them with a distribution in several types of vegetation and specific hosts (Döbbeler 2002). Despite the ecological importance of these fungi, Stenroos et al. (2010) reported that some aspects of phylogenetic diversity, life cycles, infection mechanisms, and nutrition strategies are still unknown. Seven genera of the Pyronemataceae (Pezizales) have been described associated with different moss species: *Lamprospora* De Not., *Leucoloma* Fuckel, *Neottiella* (Cooke) Sacc., *Octospora* Hedw., *Octosporella* Döbbeler and *Octosporopsis* U. Lindem & M. Vega (Stenroos et al. 2010; Németh et al. 2017; Uzun et al. 2018; Quintero et al. 2020; Sochorová et al. 2020). From Mexico, there are two records: *Octospora leucoloma* Hedw. [*Humaria leucoloma* (Hedw.) Sacc.], cited from Veracruz (Chacón and Medel 1992) and *Neottiella rutilans* (Fr.) Dennis from Michoacán (Díaz-Barriga et al. 1988); the last lacks a description. Here, we identify and describe three species of bryosymbiotic fungi new to Mexico: *Neottiella albocincta* (Berk. & M.A. Curtis) Sacc., *Octospora maireana* (Seaver) Yei Z. Wang and *Octospora texensis* Benkert, and the distribution of *Neottiella albocincta* in Mexico is expanded.

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METHODS

The specimens were collected from 2014 to 2023 in several forest types (*Pinus–Quercus*, *Quercus*, riparian, and montane cloud forest) in the states of Puebla and Tamaulipas, Mexico (Figure 1). The collections were deposited in the herbarium of the Escuela Nacional de Ciencias Biológicas at the Instituto Politécnico Nacional (ENCB), the mycological herbarium "José Castillo Tovar" from the Instituto Tecnológico de Ciudad Victoria (ITCV), and the herbarium from the Facultad de Estudios Superiores Zaragoza of the Universidad Nacional Autónoma de México (FEZA).

The specimens were characterized in situ, describing the macroscopic morphological characters, such as size, shape, and color. The *Illustrated Dictionary of Mycology* (Ulloa and Hanlin 2006) was used for

Figure 1. Map showing new localities of the four species newly reported from Mexico. Previous Mexican localities are included for two species.

mycological terminology and *Methuen Book of Color* (Kornerup and Wanscher 1978) was used for color descriptions. Longitudinal cuts of the apothecia were made, and next rehydrated in water, 70% alcohol, and 5% KOH. Melzer's reagent was used for asci reaction and cotton blue was used to observe ascospore ornamentation. Microscopically characteristics such as exciples, paraphyses, asci, and ascospores were described using an optical microscope (Axiostar plus, Zeiss, Jena, Germany). The photographs were taken with a Rebel T-1i camera, a 100mm macro lens (Canon, Tokyo, Japan), and a DCS-W630 camera (Sony, Tokyo, Japan). For scanning electron microscopy (SEM), samples were rehydrated in hot water for 10 min, fixed with 4% glutaraldehyde for 24 h, dehydrated through an ascending ethanol series, critical point dried with CO_2 (Quorum K850), sputter-coated with gold (Qurorum Q 150R ES), mounted on aluminum slides on carbon tape, and examined a Hitachi High Technologies, model SU1510 (Tokyo, Japan) to observe details of the ascospore ornamentation. No sections to verify the infection in moss were made. The identification of the specimens was carried out following the studies by Seaver (1914; 1928), Dennis and Itzerott (1973), Beug et al. (2014), and Eckstein et al. (2020). For the identification of the host genus, the *Guide to the Bryophytes of Tropical America* (Grandstein et al. 2001) was consulted.

RESULTS

***Neottiella albocincta* (Berk. & M. A. Curtis) Sacc.**, Sylloge Fungorum 8: 190 (1889)
Figures 2–4

New records. MEXICO – **Puebla** • Atempan municipality, cerro de Capitanco; 19°49′01″N, 097°27′05″W; 2064 m alt; 02.VIII.2024; M. Sánchez; MS-ENCB-16, MS-FEZA-16 • ibid.; 13.IX.2014; M. Sánchez; MS-ENCB132, MS-FEZA-132 • ibid.; 27.XI.2014; M. Sánchez; MS-ENCB-313, MS-FEZA-313 • ibid.; 25.IV.2015; A. Díaz-Pérez; ADP-FEZA-259 • ibid.; A. Díaz-Pérez; ADP-FEZA-303 • ibid.; M. Sánchez; MS-ENCB-364, MS-FEZA-364 • ibid.; 23.VII.2016; A. Díaz-Pérez; ADP-FEZA-496 • ibid.; A. Díaz-Pérez; ADP-FEZA-521 • ibid; A. Díaz-Pérez ADP-FEZA-526 • ibid.; 01.VIII.2020; M. Sánchez MS-ITCV-1973. Growing on *Atrichum* sp., in montane cloud forest.

Desription. Apothecia 3–7 mm in diameter, cupuliform, stipitate 1–3 mm, solitary to gregarious, deep orange (6A8), margin entire, setae white on the outside of the apothecia, 156–425 × 5–7 µm, widening towards the base 12–22 µm wide, wall 1–2 µm wide, 1–2 septa towards the base. Ectal excipulum 40–80 µm thick, *textura angularis*, with cells 6–18 × 6–15 µm, hyaline with abundant reddish orange pigments (7A8) in the cytoplasm, thin-walled. Medullary excipulum 200–266 µm thick with hyphae 4–11.2 µm in diameter, *textura intricata*, hyaline with abundant reddish orange pigments (7A8) in the cytoplasm. Subhymenium 70–104 µm thick. Hymenium 256–300 µm thick. Paraphyses 3–7 µm wide, filiform, hyaline with reddish orange pigments (7A8) in the cytoplasm at the apex, irregular and widening towards the apex, bifurcating towards the base. Asci 200–280 × 15–20 µm, 8-spored, uniseriate, inamyloid. Ascospores 21–23 × 12–14 µm, ellipsoid, hyaline, 1–2 guttules, reticulate or with an irregular reticulum covering the ascospore, dehiscent in 5% KOH.

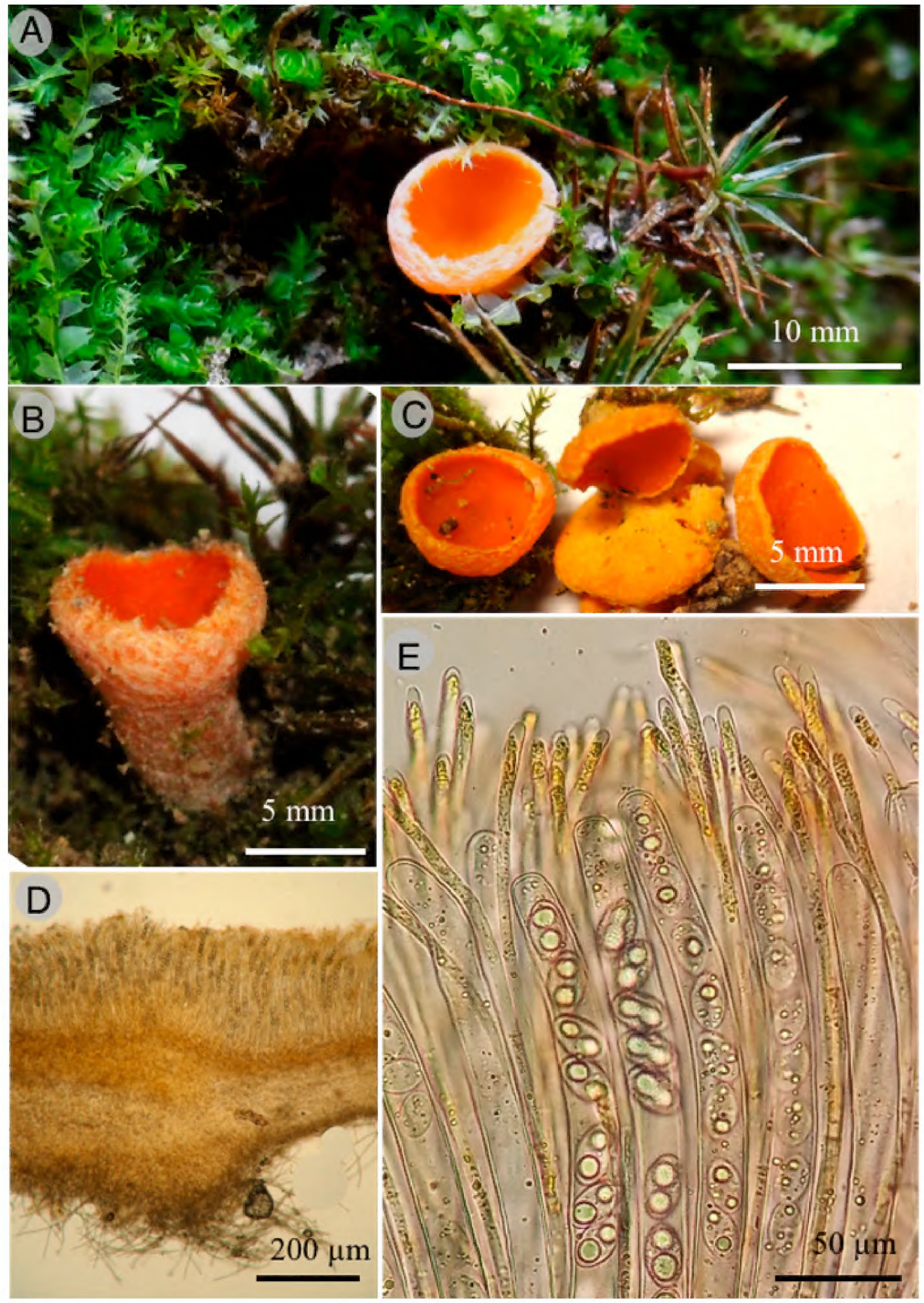
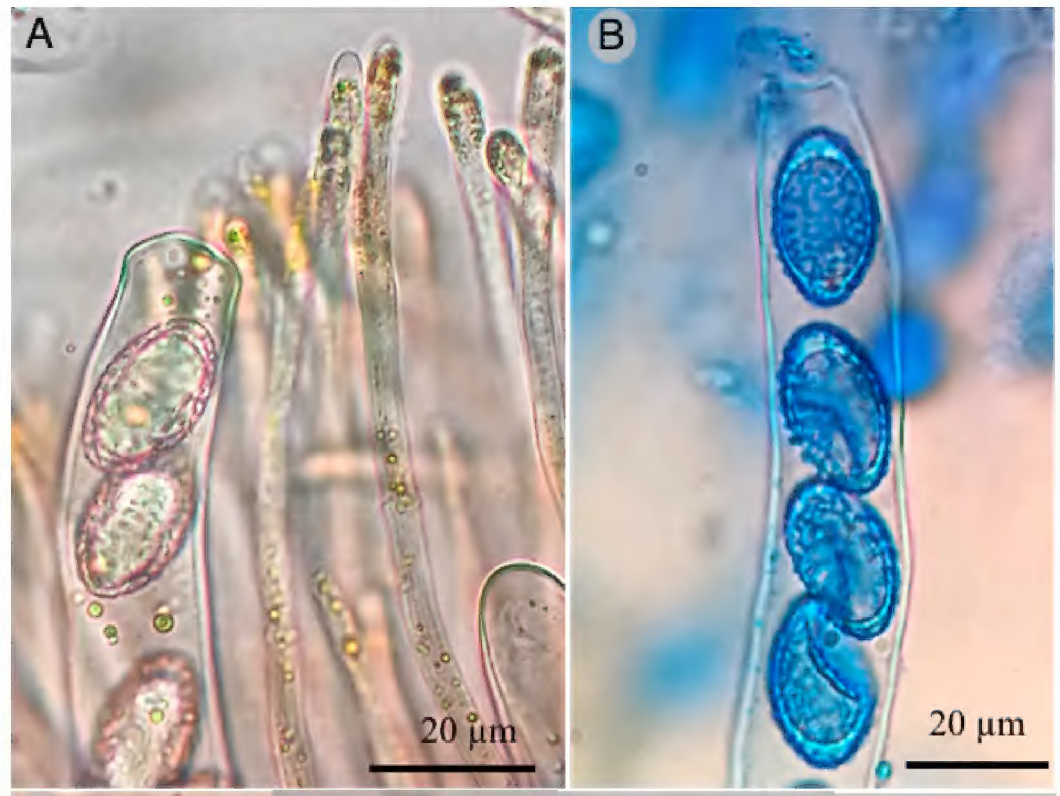


Figure 2. *Neottiella albocincta*. **A–C.** Apothecia. **D.** Longitudinal section of the apothecium. **E.** Hymenium.

Distribution. Germany (Vega 2017; Eckstein et al. 2020), United States of America (Berkeley 1875; Seaver 1928). This is the first record for Mexico, from the state of Puebla.

Taxonomic notes. This species is characterized by the cupuliform and stipitate apothecia, with reticulated ascospores of 21–23 × 12–14 µm, and grows association with *Atrichum*. Eckstein et al. (2013) reported ascospores of 19–24 × 11–13 µm and wider paraphyses of 7–11 µm. It is distinguished from *N. rutilans* (Fr) Dennis, since *N. albocincta* presents a darker apothecial color, presents more visible white hairs on its margin, and the irregularly reticulated ascospores.



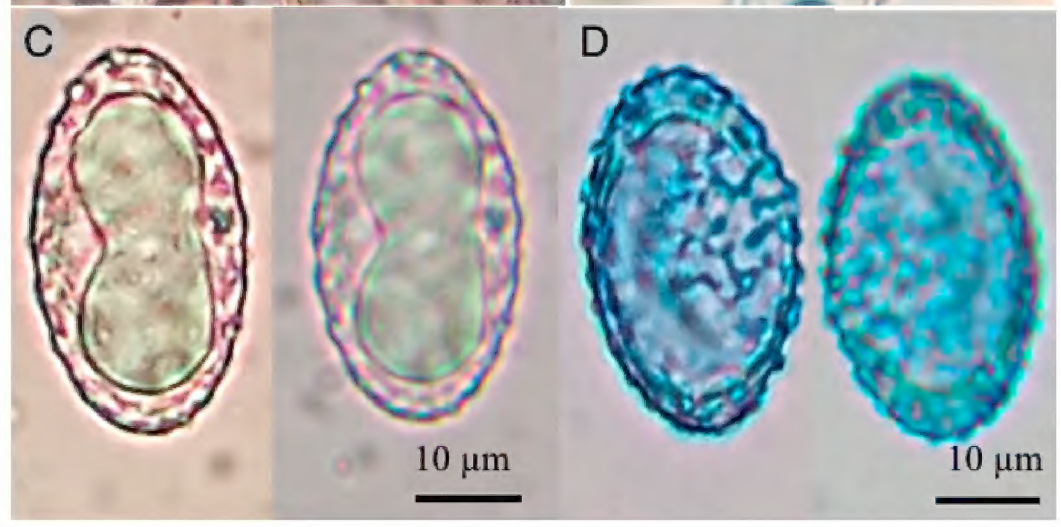
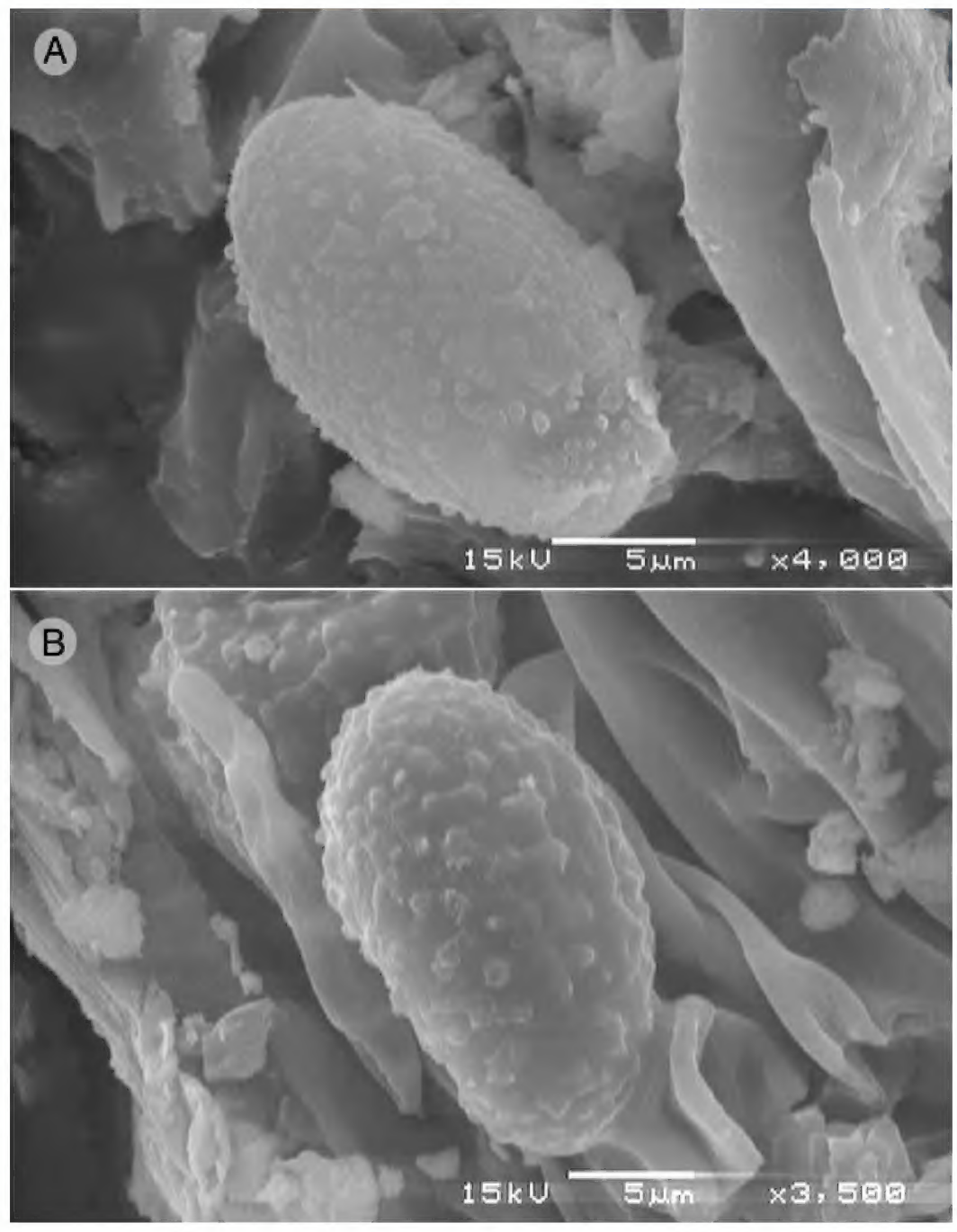


Figure 3. *Neottiella albocincta*. **A.** Asci and paraphyses. **B.** Asci and ascospores in cotton blue. **C.** Ascospores in water. **D.** Ascospores in cotton blue.

Figure 4. *Neottiella albocincta*. **A, B.** SEM of ascospores.



***Neottiela rutilans* (Fr.) Dennis**, British Cup Fungi & Their Allies: an Introduction to the Ascomycetes 28. 1960.
Figures 5–7

New records. MEXICO – **Puebla** • Honey municipality, Rincón de Chila, cascadas Arcoíris; 20°15′32.4″N, 098°14′48.3″W; 2144 m alt; 20.XI.2021; M. Sánchez; MS-ENCB-2671, MS-ITCV-2671 • ibid.; 28.XII.2021, M. Sánchez; MS-ITCV-2387 • ibid.; M. Sánchez; MS-ITCV-2400 • ibid.; 7.II.2023; M. Sánchez; MS-ITCV-3120 • ibid.; 29.VIII.2023; M. Sánchez; MS-ITCV-3255. Growning on *Polytrichum* sp. Hedw., in montane cloud forest.

Description. Apothecia 2–4 mm in diameter, cupuliform, stipitate 1–2 mm high, gregarious, reddish orange (6A7), margin entire to crenate, with white setae on the outside of the apothecia 201–416 × 7–9 µm, wall 1–1.5 µm, 0–2 septa towards the base, filiform. Ectal excipulum 85–150 µm thick with cells 12–46 × 11–21 µm, *textura angularis*, hyaline thin-walled. Medullary excipulum 45–190 µm thick with hyphae 3–10 µm in diameter, *textura intricata*, hyaline with abundant reddish orange pigments (7B8) in the cytoplasm. Subhymenium 45–65 µm thick, with reddish orange pigments (7B8) in the cytoplasm. Hymenium 315–340 µm thick. Paraphyses 4–6 µm wide, filiform, hyaline, with rounded apex, with reddish orange pigments (7B8) in

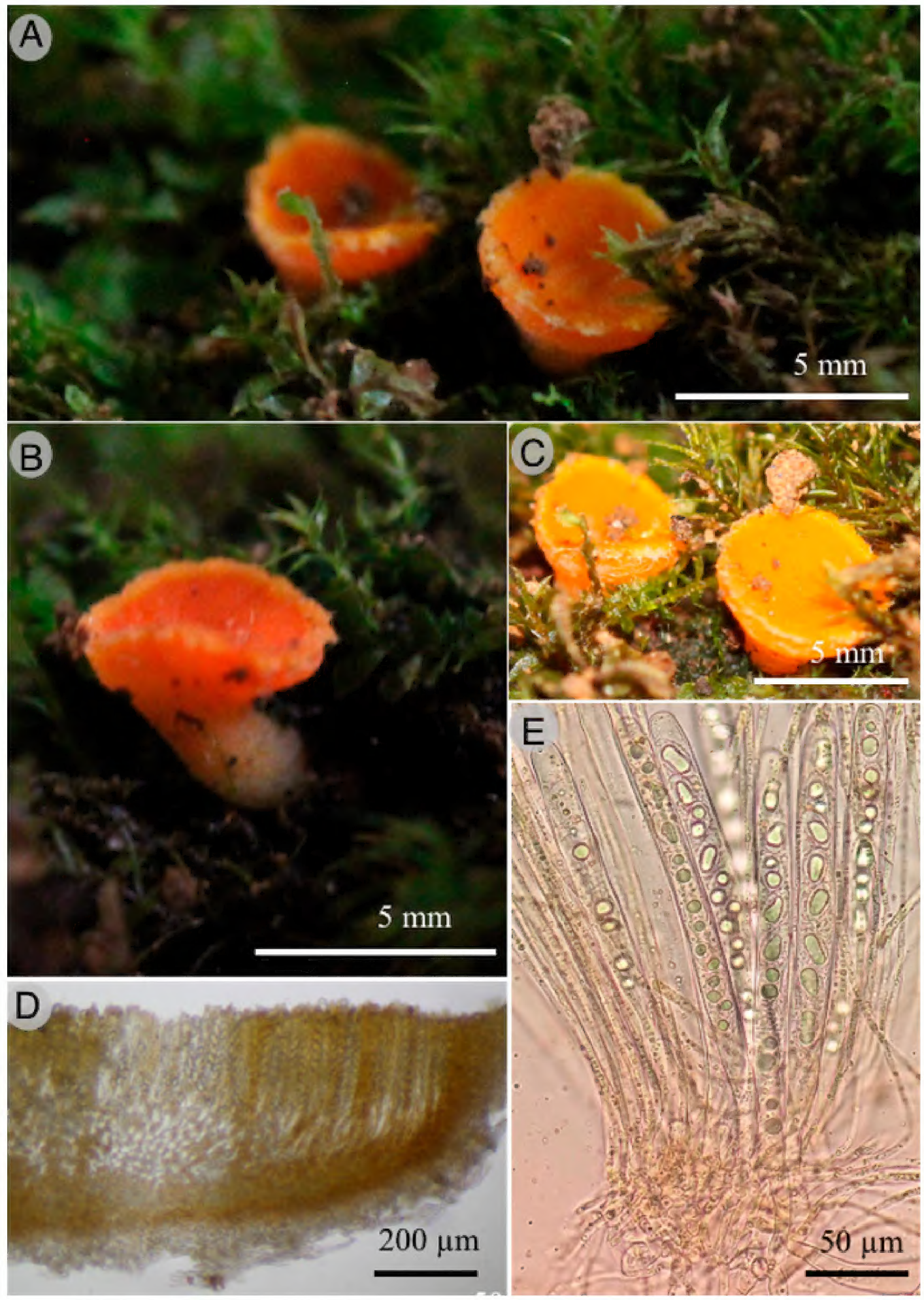


Figure 5. *Neottiella rutilans*. **A–C.** Apothecia. **D.** Longitudinal section of the apothecium. **E.** Hymenium.

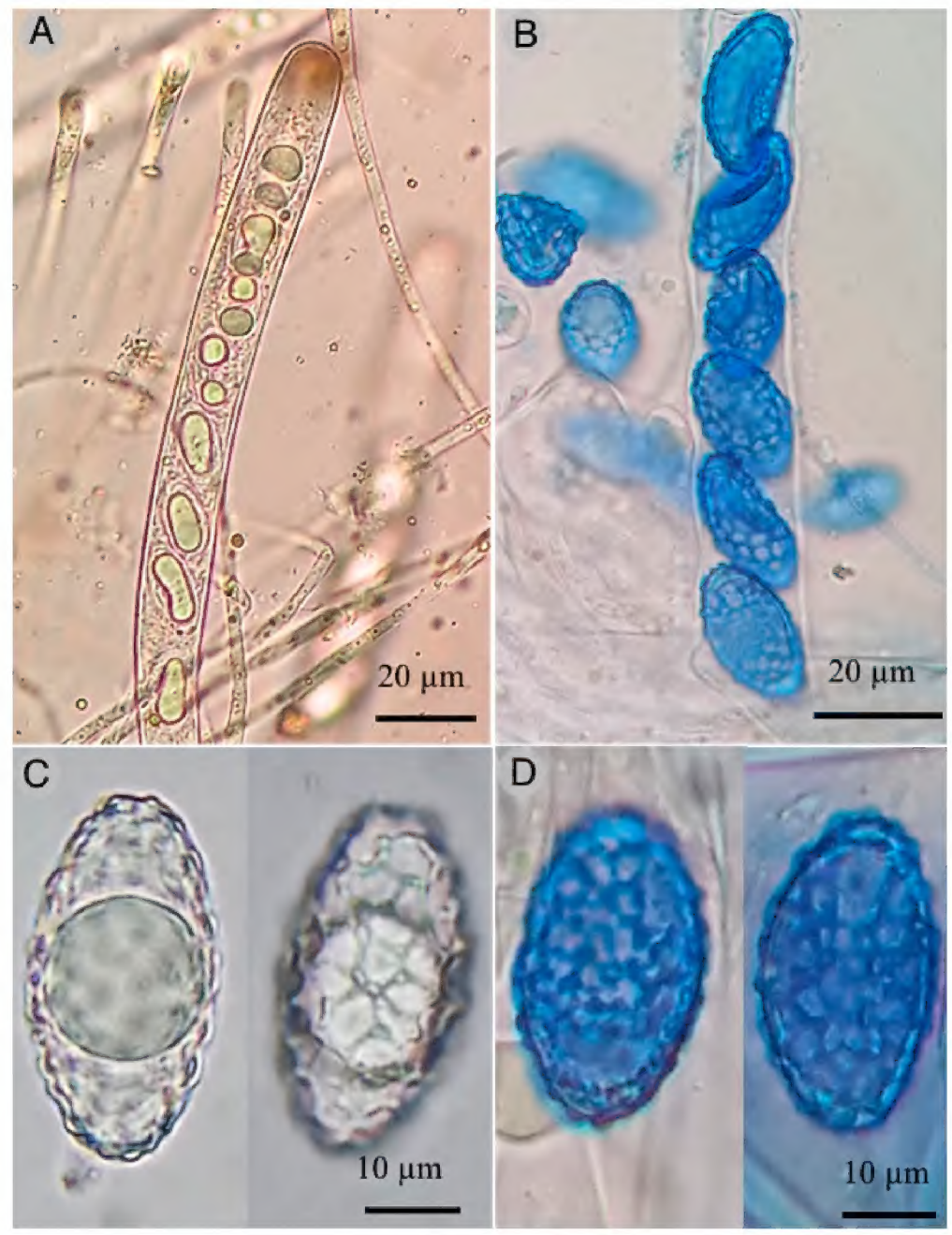


Figure 6. *Neottiella rutilans*. **A.** Asci and paraphyses. **B.** Asci and ascospores in cotton blue. **C.** Ascospores in water. **D.** Ascospores in cotton blue.

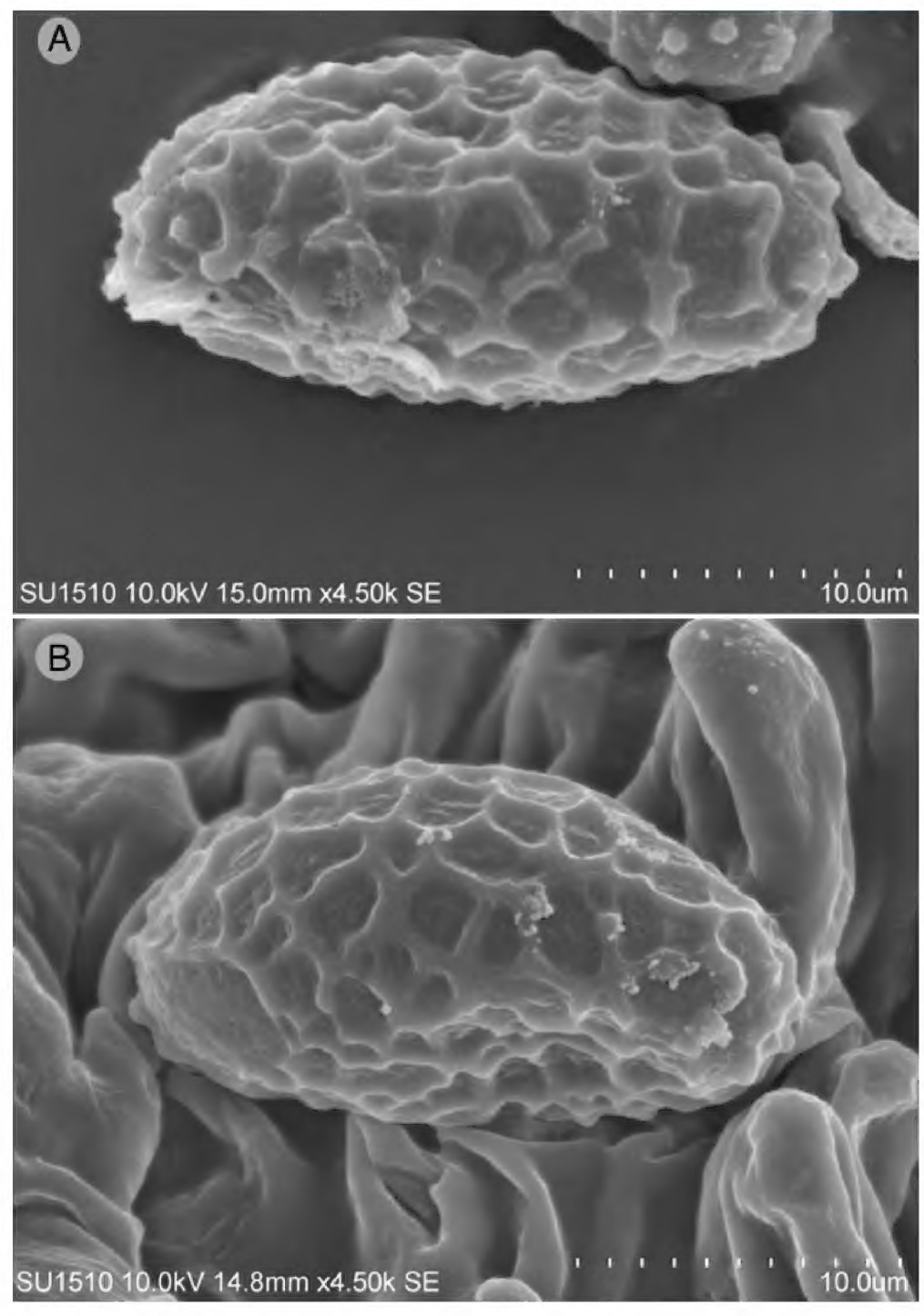


Figure 7. *Neottiella rutilans*. **A, B.** SEM of ascospores.

the cytoplasm. Asci 240–318 × 17–23(–25) µm, 8-spored, uniseriate, inamyloid. Ascospores (19–)23–27 × 12–15 µm, ellipsoid, hyaline, 1–2 guttules, reticulate throughout the ascospore, disoloving in 5% KOH.

Distribution. Germany (Vega 2017; Eckstein et al. 2020), United States of America (Beug et al. 2014), England (Yao and Spooner 1996), Turkey (Akata and Kaya 2013), and Mexico, Michoacan (Díaz-Barriga et al. 1988). It is the first record from the state of Puebla.

Taxonomic notes. This species is characterized by the cupuliform apothecia, reticulate ascospores of 23–27 × 12–15 µm, and its association with *Polytrichum*. Our description agrees with Akata and Kaya (2013) and Yao and Spooner (1996) but with shorter ascospores. It differs from *N. albocincta* by the ascospore size (21–23 × 12–14 µm) and the association that species with *Atrichum*. *Neotiella vivida* (Nyl.) Dennis shows similar ascospore size but verrucose ascospore ornamentation (Eckstein et al. 2014).

***Octospora maireana* (Seaver) Yei Z. Wang**, Special Publication of the National Museum of Natural Science 4: 41. 1992.
Figures 8–10

New record. MEXICO – **Tamaulipas** • Victoria municipality, Puerto El Paraiso community; 23°31′38.99″N, 099°12′20.04″W; 1650 m alt; 01.XI.2019; M. Sánchez; 1840 MS-ITCV-1840. Growing on *Archidium* sp Brid., in *Quercus* forest.

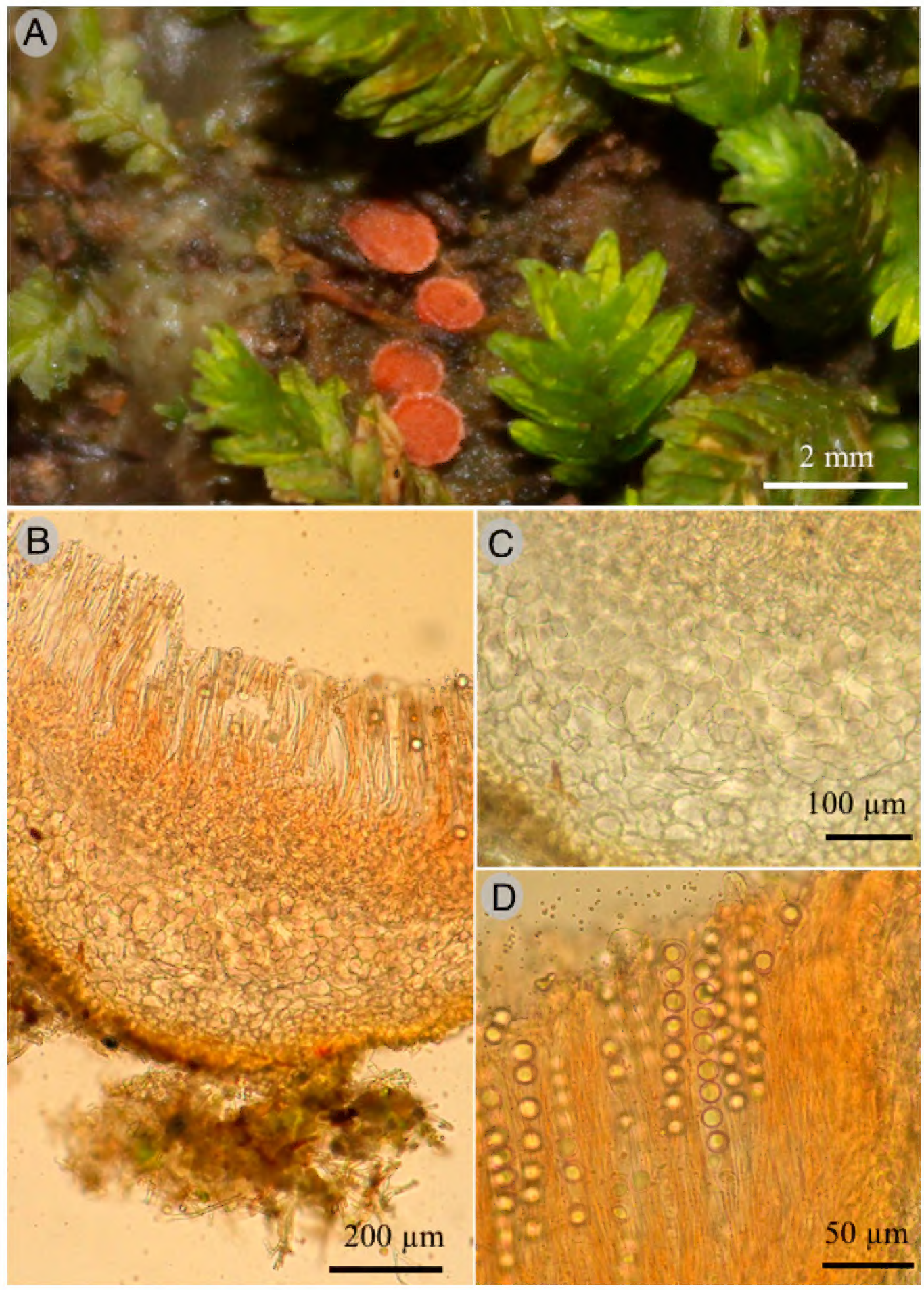


Figure 8. *Octospora maireana*. **A.** Apothecia. **B.** Longitudinal section of the apothecium. **C.** Ectal excipulum. **D.** Hymenium.

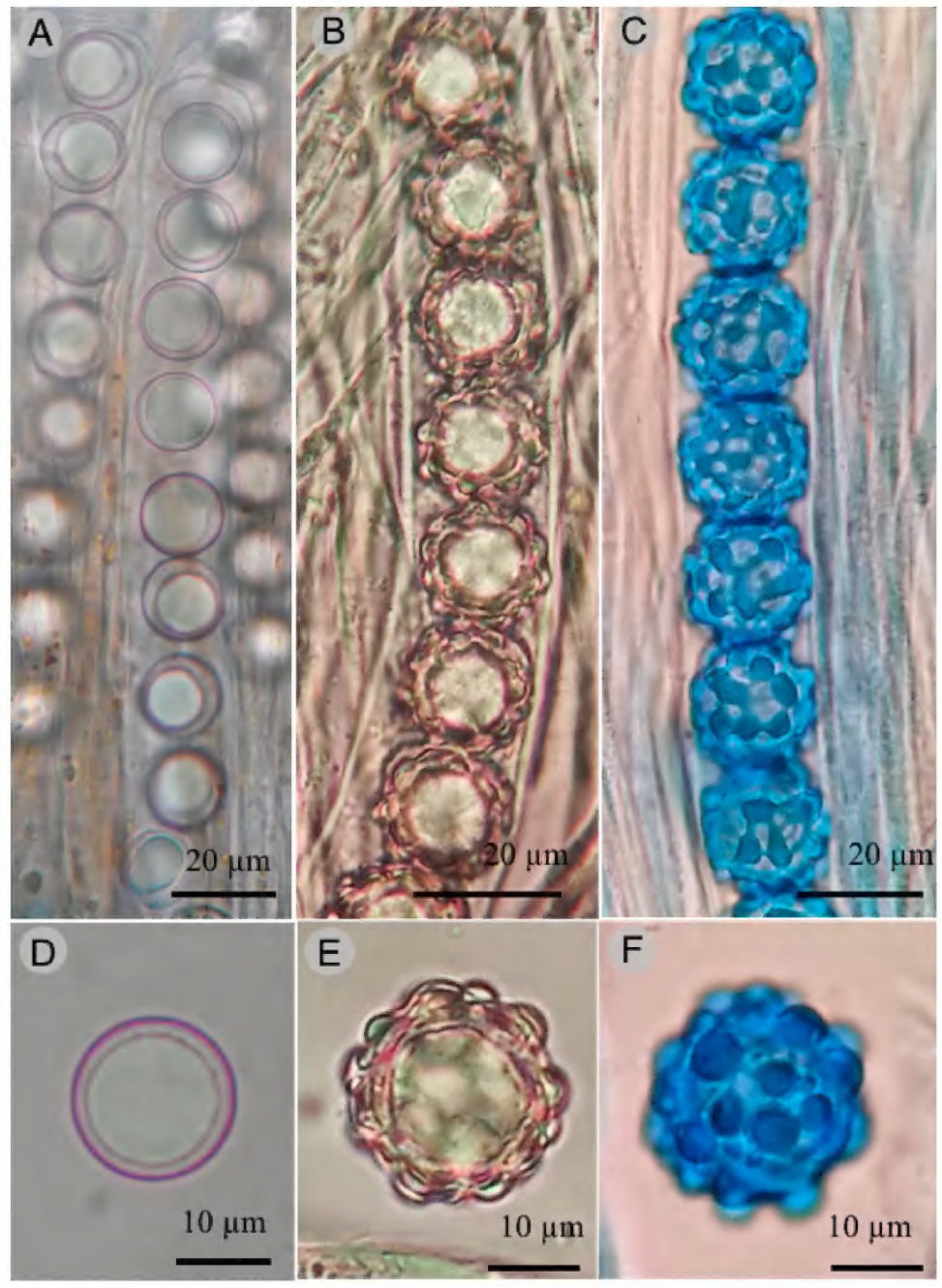


Figure 9. *Octospora maireana*. **A.** Asci and ascospores in 5% KOH. **B.** Asci and ascospores in water. **C.** Asci and ascospores in cotton blue. **D.** Ascospore in 5% KOH. **E.** Ascospore in water. **F.** Ascospore in cotton blue.

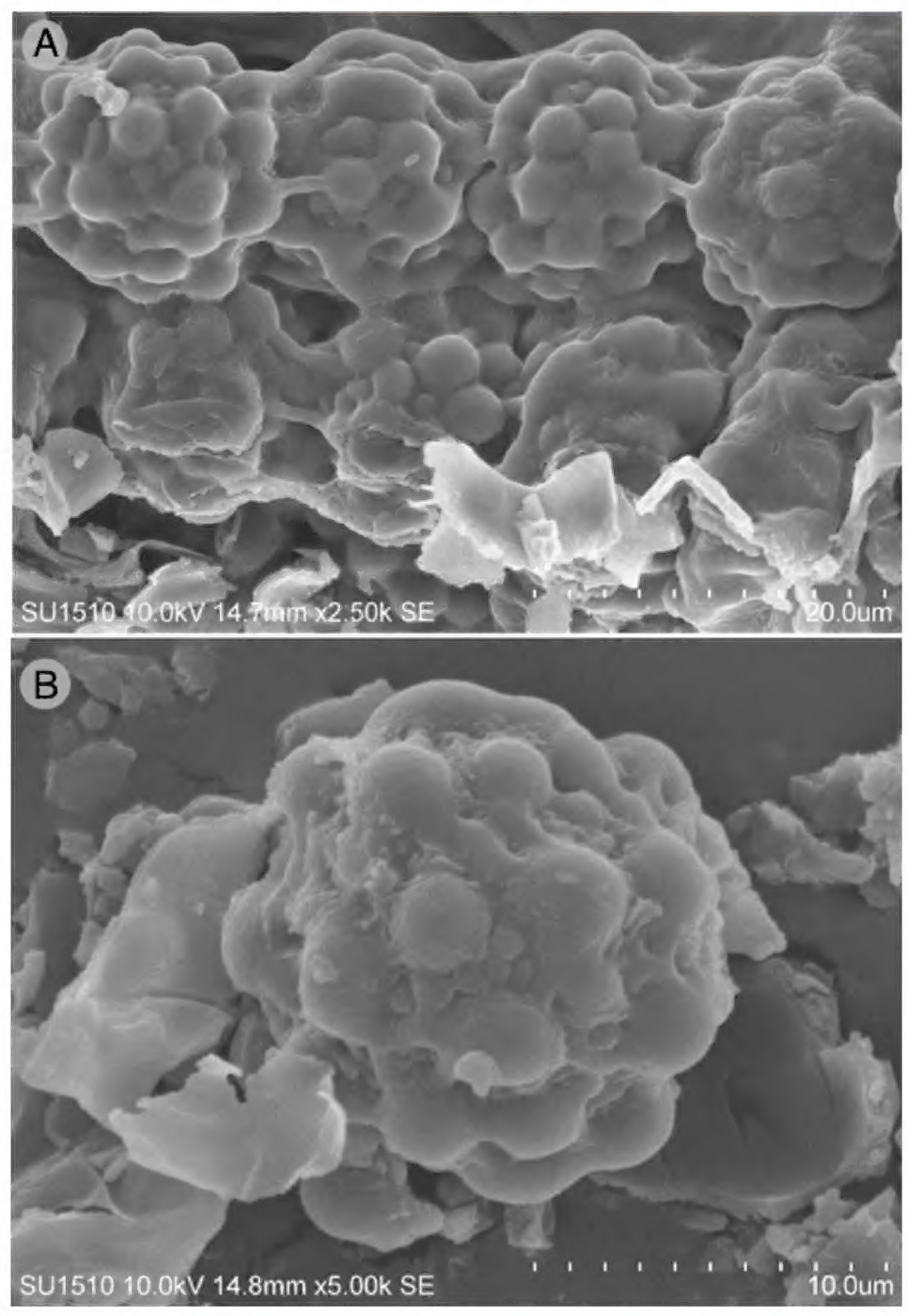


Figure 10. *Octospora maireana*. **A.** Asci and ascospores on SEM. **B.** SEM of ascospores.

Desription. Apothecia 0.5–1 mm in diameter, discoid, sessile, solitary to gregarious, reddish orange (7A7), margin crenate, pastel red (7A5). Ectal excipulum 75–212 µm thick with cells 12–42 × 8–26 µm, *textura angularis*, subhyaline, thin-walled. Medullary excipulum 15–38 µm thick with hyphae 4–7 µm in diameter, *textura intricata*, subhyaline. Subhymenium 35 µm thick. Hymenium 290–380 µm thick. Paraphyses 4–5 µm wide, filiform, salmon (6A4) in the cytoplasm, rounded at the apex, bifurcating towards the base. Asci 250–269 × 18–25 µm, cylindrical, 8-spored, uniseriate, inamyloid. Ascospores 13–16 µm in diameter excluding ornamentation, globose, hyaline, with a guttula that fills almost the entire ascospore, tuberculate, 2–3 µm high, 3–5 µm wide, dehiscent in 5% KOH.

Distribution. Germany (Eckstein et al. 2020), Algeria (Seaver 1914), Australia (Rifai 1968), Finland (Kullman 1997; Jakobson et al. 1998), Portugal (Ortega and Buendía 1987), United States of America (Seaver 1928). This is the first record for Mexico, from the state of Tamaulipas.

Taxonomic notes. This species is characterized by the orange discoid apothecia, ascospores 13–16 µm, and the association with *Archidium*. It differs from *O. tuberculata* (Seaver) Caillet & Moyne by the yellow to orange apothecia and the ascospore size (14–16 µm) and having a strong tuberculate ornamentation (Seaver 1912; Eckstein et al. 2014; Egertová et al. 2015). The Mexican specimen agrees with the description of Benkert (1987), but it has slightly larger ascospores (15–20 µm). Seaver (1914) and Kullman (1997) reported larger ascospores of up to 23 µm and 18.9–21.4 µm diameter, respectively. These authors observed the presence of a pseudostipe; our Mexican specimens lack this feature. Furthermore, the specimens described by Kullman (1997) have a more crowded ornamentation.

***Octospora texensis* Benkert**, Zeitschrift für Mykologie 64 (1): 28. 1998.
Figures 11–13

New record. MEXICO – Tamaulipas • Victoria municipality, cañón del Novillo; 23°41′44.59″N, 099°12′20.63″W; 436 m alt; 07.XI.2019; M. Sánchez; MS-ITCV-1859 • ibid.; MS-ITCV-1860. Unidentified moss, in riparian forest.

Description. Apothecia 1–4 mm in diameter, discoid, sessile, solitary to gregarious, reddish orange (7B8), margin entire. Ectal excipulum 125–188 µm thick with cells 47–110 × 27–90 µm, *textura angularis*, hyaline, thin-walled, setae 65–183 × 8–14 µm, walls 1–3.5 µm thick, light brown (6D8) subhyaline, acute at the apex, sometimes with a septum. Medullary excipulum 33–100 µm thick with hyphae 5–10 µm wide, *textura intricata*, subhyaline. Subhymenium 20–33 thick. Hymenium 275–295 µm thick. Paraphyses 7–9 µm wide, filiform, hyaline, with reddish orange (7B8) pigments in the cytoplasm, widening towards the apex, septate. Asci 226–256 × 15–21 µm, cylindrical, 8-spored, some forming only four mature ascospores with four immature ones, uniseriate, inamyloid. Ascospores 15–18 × 10–12 µm not including ornamentation, ellipsoid, hyaline, with one guttule that encompasses most of the ascospore, ornate 1–2.5 µm high, 1–3.5 µm wide, tuberculate, dehiscent in 5% KOH.

Distribution. United States of America (Benkert 1998). From the state of Tamaulipas, this is the first record for Mexico.

Taxonomic notes. This species is characterized by the orange apothecia and tuberculate ascospores of 15–18 × 10–12 µm. Benkert (1998) reported ascospores of a similar size (16–18 × 12.5–15 µm), growing in association with *Schwetschkeopsis fabronia* (Schwärg.) Broth.; however, due to the lack of diagnostic structures, the host moss of our specimens could not be identified. *Octospora phagospora* (Flageolet & Lorton) Dennis & Itzerott is a similar species with similar ascospore size (16–18 × 11–12 µm), and lacks setae on the receptacle (Dennis and Itzerott 1973). Our species also differs from *O. rustica* (Velen.) J. Moravec by the paler apothecia and slightly more slender smooth ascospores (7.6–11.9 µm) (Billekens 1992). *Octospora kilimanjarensis* is a similar species, as it has hairs on its ectal excipulum; however these are larger (70–220 × 6–12 µm), as are its ascospores (18–22 × 13.5–16.5 µm) (Moravec 1997). *Octospora texensis* can be confused with *O. similis* (Kirschst.) Benkert due to the similar range of ascospore size (15–)17–18(–19) × (11–)11.5–12(–14) µm, however, the ornamentation is less pronounced in *O. similis* and their asci are octosporatous (Eckstein and Eckstein 2013).

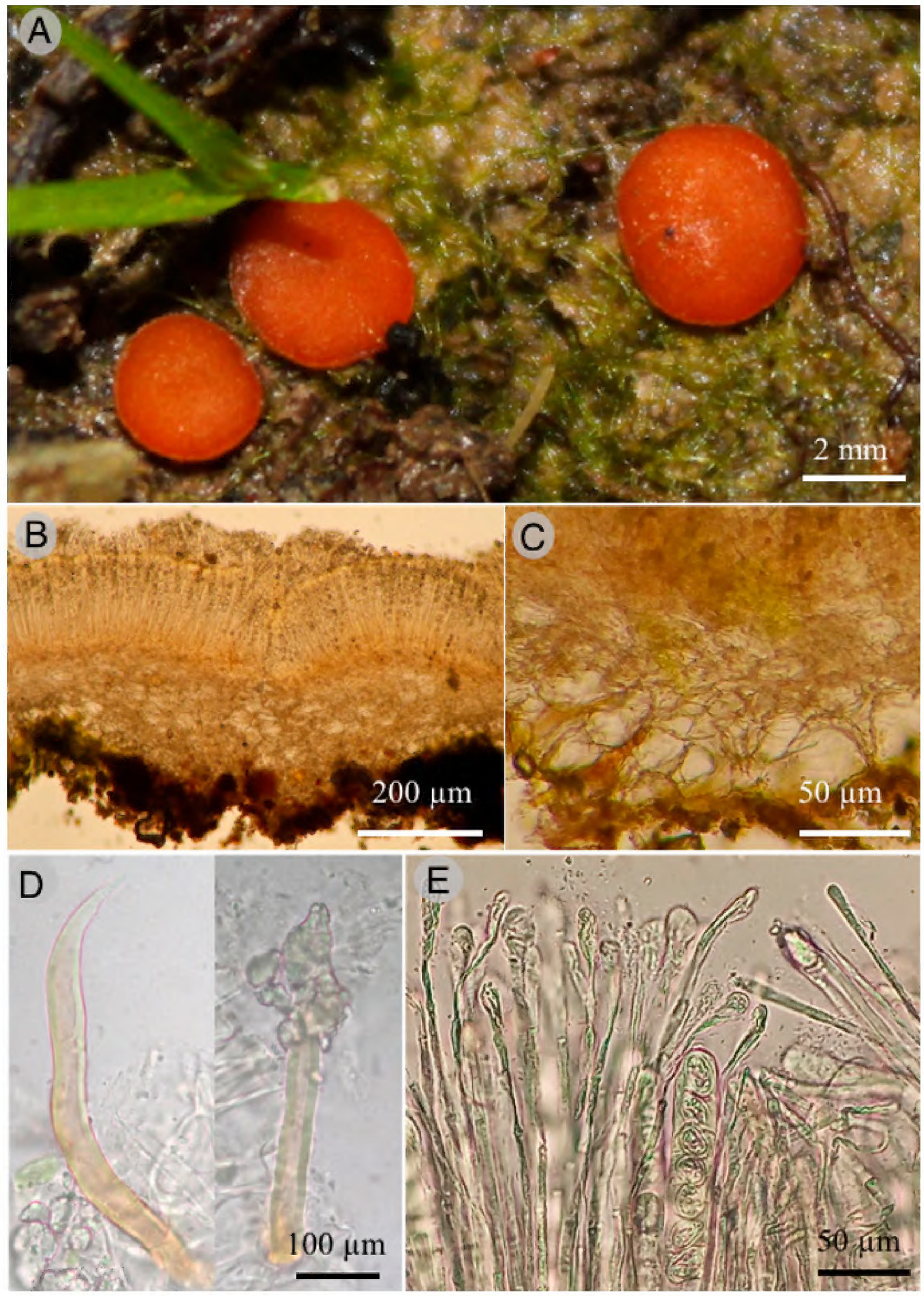


Figure 11. *Octospora texensis*. **A.** Apothecia. **B.** Longitudinal section of the apothecium. **C.** Ectal excipulum. **D.** Setae. **E.** Asci and paraphyses.

DISCUSSION

Prior to this study, *Octospora leucoloma* (described as *Humaria leucoloma*) and *Neotiella rutilans* were the only bryosymbiotic fungi known from Mexico; this is related to the scarce knowledge about the Pyronemataceae family in the country. Thus, *N. albocincta*, *O. maireana* and *O. texensis* are reported for the first time in Mexico, and the distribution *N. rutilans* is described from a new locality. Bryosymbiotics taxa such as *Lamprospora*, *Leucoloma*, *Neottiella*, *Octospora*, *Octosporella*, and *Octosporopsis* are clustered in a clade characterized by forming small discoid, cupuliform, or peritecioid apothecia, and have an obligate association with bryophytes. This association has been interpreted as parasitic in nature or bryosymbiotic species (Perry et al. 2007; Hansen et al. 2013; Quintero et al. 2020). If we consider that Mexico could harbor about 1591 species of mosses, the diversity of bryosymbiotic fungi could increase considerably among ascomycetes and basidiomycetes (Delgadillo-Moya 2014; Hernández-Rodríguez and Delgadillo-Moya 2021). We conclude that mosses could be an interesting habitat to continue and expand studies for this group of fungi in the country. However, in the case of *O. texensis*, it was not possible to identify its host because in the locality where it was collected it is highly disturbed by anthropogenic actions and the mosses have been scarce. More taxonomic, molecular, and cytological studies on this little-studied group of fungi and specialized knowledge of bryophytes are recommended.

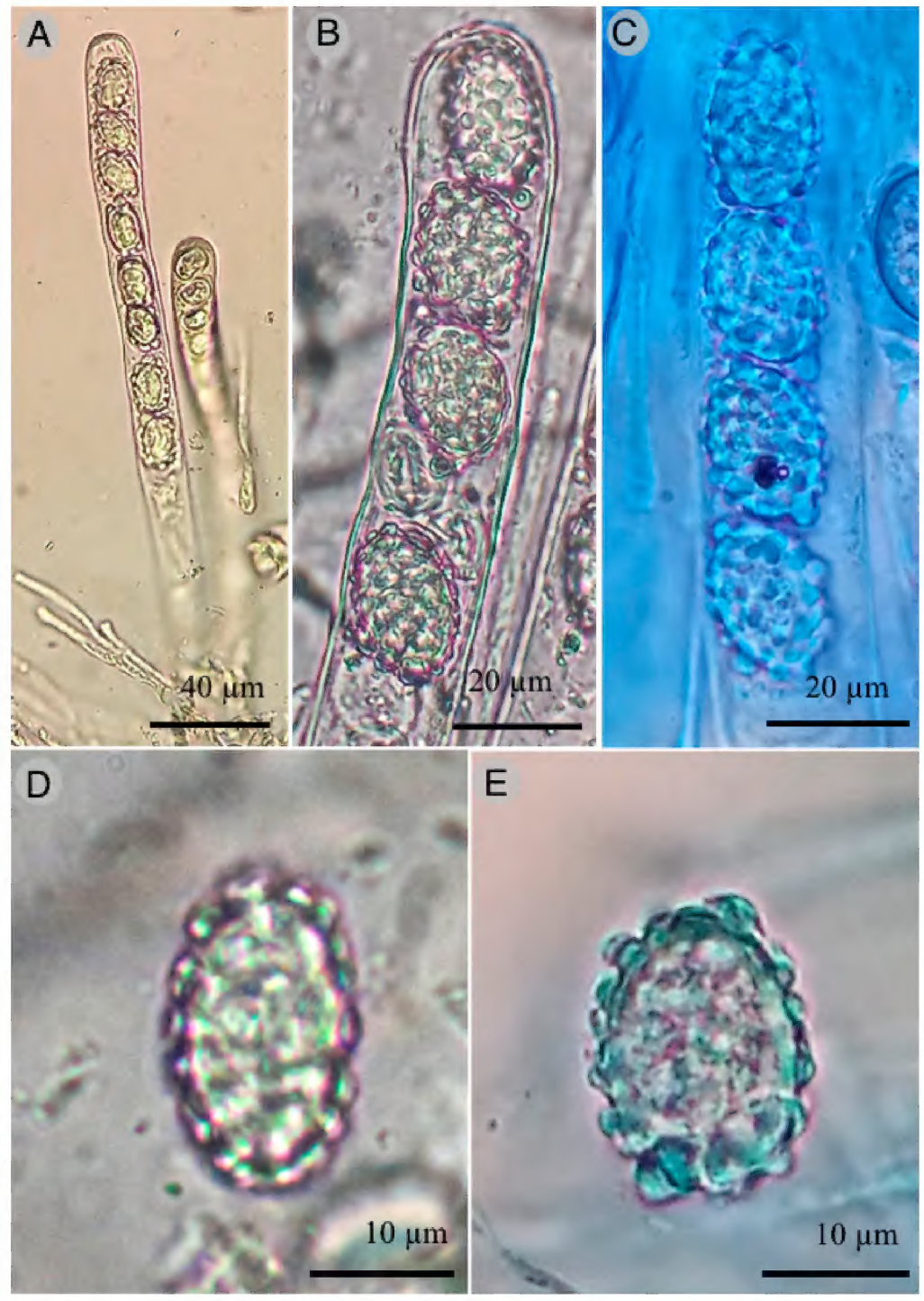


Figure 12. *Octospora texensis*. **A, B.** Asci and ascospores in water. **C.** Asci and ascospores in cotton blue. **D.** Ascospore in water. **E.** Ascospore in cotton blue.

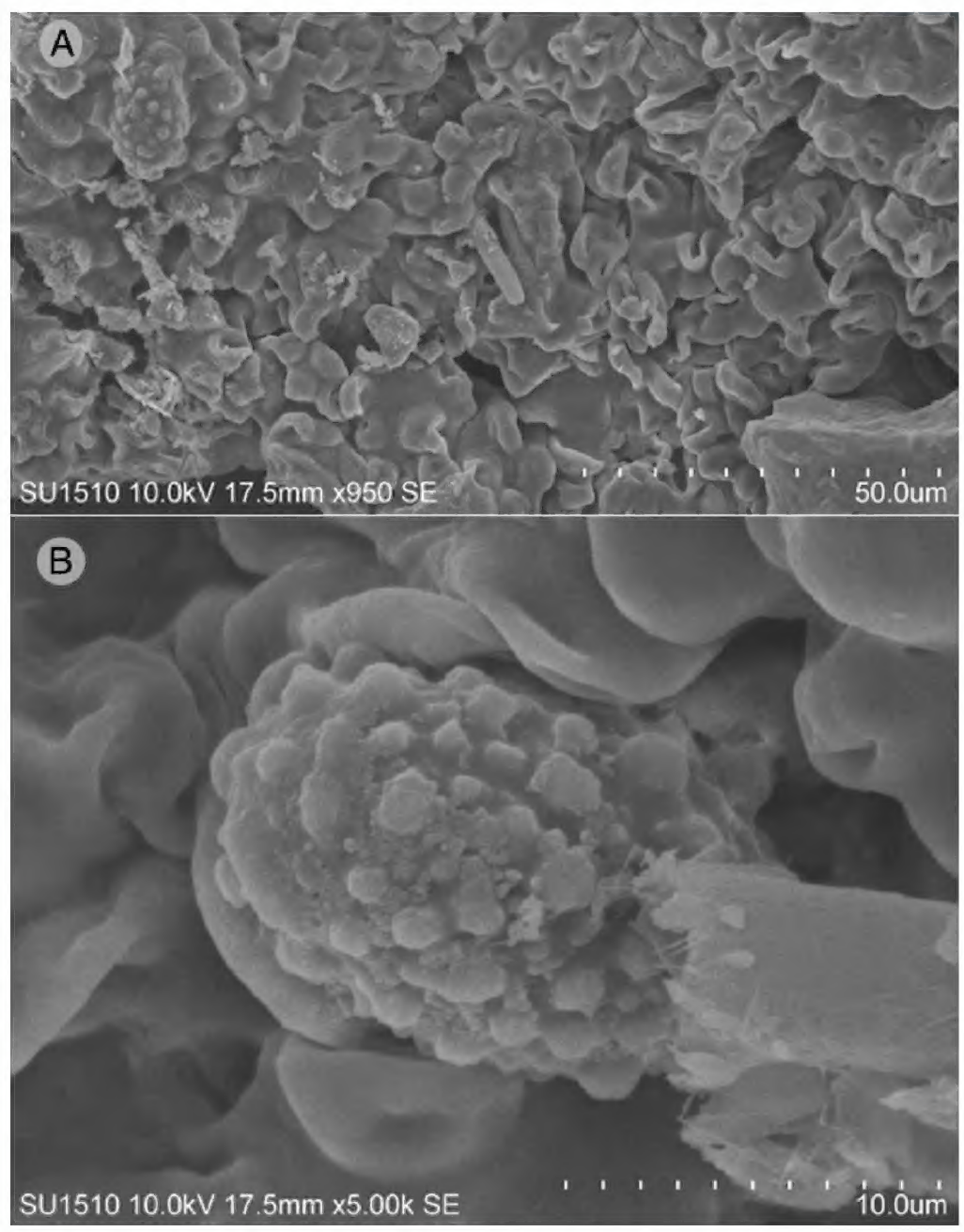


Figure 13. *Octospora texensis*. **A, B.** SEM of ascospores.

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Author contributions

Conceptualization: MSF, JGJ, TR. Fieldwork: MSF, JGJ, JFHV. Taking photographs: MSF. Rewiew of herbarium material: MSF. Identifying species: MSF. Takin g photographs on SEM: MBMG. Recompilation of information: MSF, JGJ, JIF, TR. Making figures: TR. Writing, reviewing, and editing: MSF, JGJ, JIF, JFHV, MBMG, TR.

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Data availability

All data that support the findings of this study are available in the main.

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